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Sex differences in the hypothalamus in the different stages of human life☆

Dick F. Swaab[a,*], Wilson C.J. Chung[a], Frank P.M. Kruijver[a], Michel A. Hofman[a], Andon Hestiantoro[a,b]

[a] *Graduate School Neurosciences Amsterdam, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands*
[b] *Department of Obstetrics and Gynaecology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia*

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Abstract

Quite a number of structural and functional sex differences have been reported in the human hypothalamus and adjacent structures that may be related to not only reproduction, sexual orientation and gender identity, but also to the often pronounced sex differences in prevalence of psychiatric and neurological diseases. One of the recent focuses of interest in this respect is the possible beneficial effect of sex hormones on cognition in Alzheimer patients. The immunocytochemical localization of estrogen receptors (ER) α, β and androgen receptors has shown that there are indeed numerous targets for sex hormones in the adult human brain. Observations in the infundibular nucleus have, however, indicated that in this brain area the hyperactivity resulting from a lack of estrogens in the menopause seems to protect females against Alzheimer changes, in contrast to males. It is thus quite possible that estrogen replacement therapy may, in these brain areas, lead to inhibition of neuronal metabolism and thus to the same proportion of Alzheimer changes as are observed in men. Knowledge about the functional sex differences in the brain and the effect of sex hormones on neuronal metabolism may thus provide clues not only for the possible beneficial effects of these hormones (e.g., on cognition or hypertension), but also on possible central side effects of estrogen replacement therapy.

Keywords: Sex differences; Hypothalamus; Alzheimer patients; Estrogen receptors; Estrogen replacement therapy

Sex differences in the brain may be the basis not only for sex differences in reproduction, gender identity (the feeling to be male or female) and sexual orientation (heterosexuality, homosexuality), but also for sex differences in prevalence of psychiatric and neurological diseases in adulthood and in age-related neurodegeneration. The proportions of cases range from more than 75% women in Rett syndrome, lymphocytic hypophysitis, anorexia and bulimia nervosa and hypnic headache syndrome, to more than 75% men in dyslexia, ADHD, autism, sleep apnoea, Gilles de la Tourette syndrome, rabies, Kallman syndrome and Kleine–Levin syndrome (Table 1). Whether sex differences in the brain that arise in development ("organizing effects") are indeed the basis for the sex difference in neurological or psychiatric diseases has yet to be established. In ADHD, an association with androgen receptor haplotypes was found [23]. An alternative mechanism for the sex differences in prevalence for brain disorders are the immediate effects of circulating sex hormone levels ("activating effects") as shown in, for example, sleep apnoea. In this review, we discuss a few examples of structural and functional sex differences in the human hypothalamus in the different stages of life.

1. Structural sex differences

Structural sex differences have been reported in a number of human hypothalamic nuclei ([95]; Figs. 1 and 2) but the data from these studies are still controversial. Sex difference in the sexually dimorphic nucleus of the preoptic area (SDN-POA) that was first described in the rat by Gorski et al. [41] and is three to eight times larger in male rats than in female rats, is so evident that it can even be observed with the naked eye in Nissl-stained sections. We have found a sexually dimorphic nucleus in the preoptic area of the human hypothalamus ([49,99,100]; Figs. 1 and 2) that we presume to be homologous to the SDN-POA in the rat as judged from its sex difference in young adults in size and cell number (Fig. 3), localization, cytoarchitecture and neurotransmitter/neuromodulator content. Immunocytochemical studies support such a homology between the SDN-POA in rat and

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* Corresponding author. Tel.: +31-20-5665500; fax: +31-20-6961006.
E-mail address: d.swaab@nih.knaw.nl (D.F. Swaab).

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Table 1
Ratios for women over men suffering from a selection of neurological and psychiatric diseases

Disease	Percent women: percent men
Rett syndrome	100:0
Postoperative hyponatremic encephalopathy with permanent damage or death	96:4
Anorexia nervosa	93:7
Lymphocytic hypophysitis	90:10
True (central) precocious puberty	90:10
Hypnic headache syndrome	84:16
Bulimia	75:25
Senile dementia of the Alzheimer type	74:26
Multiple sclerosis	67:33
Anxiety disorder	67:33
Posttraumatic stress disorders	66:34
Dementia	64:36
Unipolar depression, dysthymia	63:37
Whiplash	60:40
Severe learning disability	38:62
Substance abuse	34:66
Stuttering	29:71
Schizophrenia	27:73
REM sleep behavioral disorder	24:76
Male-to-female versus female-to-male transsexuals	28:72
Dyslexia	23:77
ADHD	20:80
Autism	20:80
Sleep apnoea	18:82
Kallmann syndrome	17:83
Rabies	13:87
Gilles de la Tourette	10:90
Kleine–Levin syndrome	0:100

For references see [98].

human on the basis of the presence of thyrotropin-releasing hormone, cholecystokinin, galanin and glutamic acid decarboxylase (for review see [97]). Allen et al. [3] gave this nucleus another name: "Interstitial Nucleus of the Anterior Hypothalamus 1 (INAH-1)". Morphometric analysis of the human SDN-POA revealed that the volume is more than twice as large in young adult men as it is in women, and contains about twice as many cells in men [99]. The magnitude of the SDN-POA sex difference does not remain constant throughout adulthood, but varies with age (Fig. 4). We extended the original observations to a group of 38 females and 42 males [49,100] replicating the sex difference in the young adult group. However, neither Allen et al. [3], LeVay [67] or Byne et al. [14] found a sex difference in the SDN-POA/INAH-1.

Allen et al. [3] described two other cell groups (INAH-2 and -3; Fig. 1) in the preoptic-anterior hypothalamic area of humans that were larger in the male brain than in the female brain. It is unclear which nuclei in the rat are homologous to the INAH-2 and -3 which is further hampered by the lack of knowledge about their neurotransmitter content. Neither the data from the study by LeVay [67] nor those of Byne et al. [14] confirmed the sex difference in INAH-2, but they both did confirm a sex difference in INAH-3.

Another sex difference was described by Allen and Gorski [4] in what they called the "darkly staining posteromedial component of the bed nucleus of the stria terminalis" (BNST-dspm). The volume of the BNST-dspm was 2.5 times larger in males than in females. We found a similar sex difference in the central nucleus of the bed nucleus of the stria terminalis (BSTc; Fig. 5). The BSTc is defined by its dense vasoactive intestinal polypeptide (VIP) innervation and is characterized by its somatostatin fiber plexus and neurons. The BSTc in men is 40% larger than in women and men have almost twice as many somatostatin neurons as women [19,59,117].

The anterior commissure was found to be 12% larger in females, and the interthalamic adhesion (or massa intermedia), a grey structure that crosses the third ventricle between the two thalami, was present in more females (78%) than males (68%). Among subjects with a massa intermedia, the structure was on average 53% larger in females than in males [5]. The latter observations suggest a greater connectivity between the cerebral hemispheres of women as compared to men.

2. Development and sexual differentiation

Sexual dimorphism does not seem to be present in the human SDN-POA at the time of birth. At that moment, total cell numbers are still similar in boys and girls and the SDN-POA contains no more than some 20% of the total cell number found between 2 and 4 years of age. From birth up to this age, cell numbers increase equally rapidly in both sexes (Fig. 6). A sex difference in the SDN-POA does not occur until about the fourth year postnatally, when cell numbers start to decrease in girls, whereas in boys the cell numbers in the SDN-POA remain stable until their rapid decrease at approximately 50 years of age. In women, a second phase of marked cell loss sets in after the age of 70 ([49,100]; Fig. 4).

The sex difference in size of the BSTc becomes overt only in adulthood [19]. The unexpectedly late sexual differentiation of the BSTc shows that the process of sexual differentiation of the brain may extend into adulthood.

The sex difference in the pattern of aging, and the fact that sexual differentiation in the human SDN-POA only occurs after the fourth year of age ([100]; Fig. 6) might explain why Allen et al. [3], who had a sample of human adults biased for aged individuals, did not find a significant sex difference in the size of the SDN-POA, which they called INAH-1. In the study of Allen et al., 40% of the adult subjects came from the age group in which the SDN-POA sex difference is minimal compared to 29% in our study [49]. Moreover, the group of elderly subjects (over 70 years of age) was underrepresented in Allen's study: 20% compared to the 37.5% that would be a proportional distribution of all ages. In our study, 32% of the subjects belonged to this old age group. So

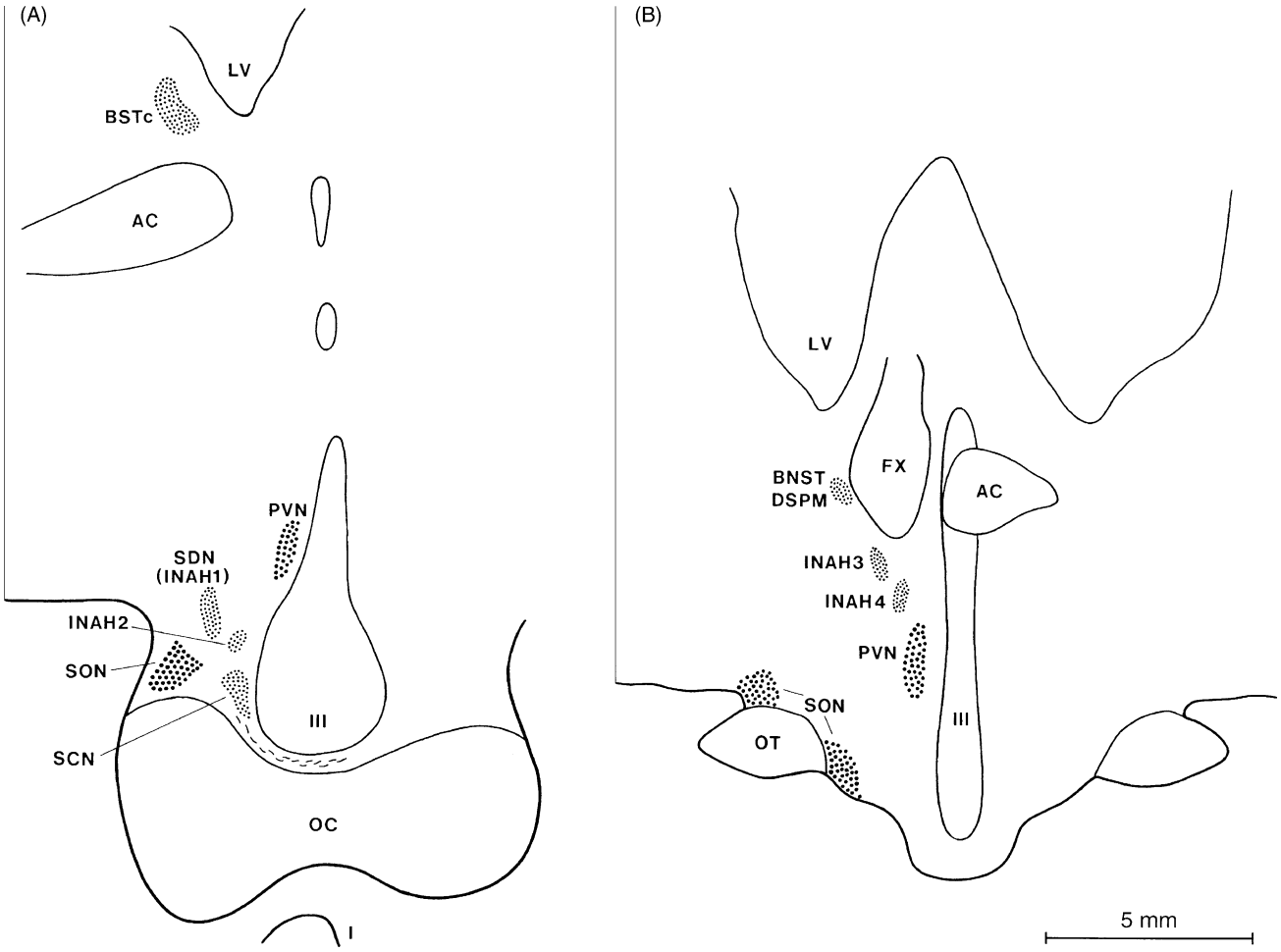

Fig. 1. Topography of the sexually dimorphic structures in the human hypothalamus: (A) is a more rostral view than (B). Abbreviations: III, third ventricle; AC, anterior commissure; BNST-DSPM, darkly staining posteriomedial component of the bed nucleus of the stria terminalis; FX, fornix; I, infundibulum; INAH1–4, interstitial nucleus of the anterior hypothalamus 1–4; LV, lateral ventricle; OC, optic chiasm; OT, optic tract; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SDN, sexually dimorphic nucleus of the preoptic area = INAH-1; SON, supraoptic nucleus. Scale bar = 5 mm. The AC, BSTc, BNST-DSPM, INAH2–4, SCN and SDN were reported to vary according to sex. The SCN, INAH3 and AC are different in relation to sexual orientation.

it seems likely that Allen et al. [3] were unable to establish a sex difference in the INAH-1 (SDN-POA) because they used a sample with a biased age distribution. A further argument for this assumption is that, if we, in our material, had studied only subjects of the age distribution of Allen's study, the sex difference in SDN-POA volume would have been reduced from a factor 2 [49] to only 1.4 times, and this difference would no longer have been statistically significant [94]. Moreover, the sex difference in the SDN-POA emerges only between the ages of 4 and puberty [100]; therefore, the brain of the 5-year-old boy and 4-year-old girl (she indeed had by far the largest volume of the entire series of female INAH-1) also produced a bias in the Allen et al. [3] study material. The age distribution, however, does not explain why LeVay [67] and Byne et al. [14] could not find a sex difference in the volume of INAH-1. Although the numbers of subjects they studied were much smaller than those in our study [100], technical differences such as section thickness or embedding method may be a possible explanation for the controversy. Anyhow, the finding that nuclear androgen (Fig. 7) and estrogen receptor (ER) α and β staining in the SDN-POA was more intense in men than in women [34,62] supports the presence of a sex difference in this nucleus.

3. The suprachiasmatic nucleus (SCN) in relation to sex and sexual orientation

3.1. Sex differences in sleep

The sex difference in the shape of the vasopressinergic SCN and in VIP-expressing cell numbers [93,101,117] suggest the possibility of sex differences in circadian patterns. Moreover, in the human SCN, nuclear androgen receptor staining was more apparent in men than in women [34], while ERα and β staining was more pronounced in women

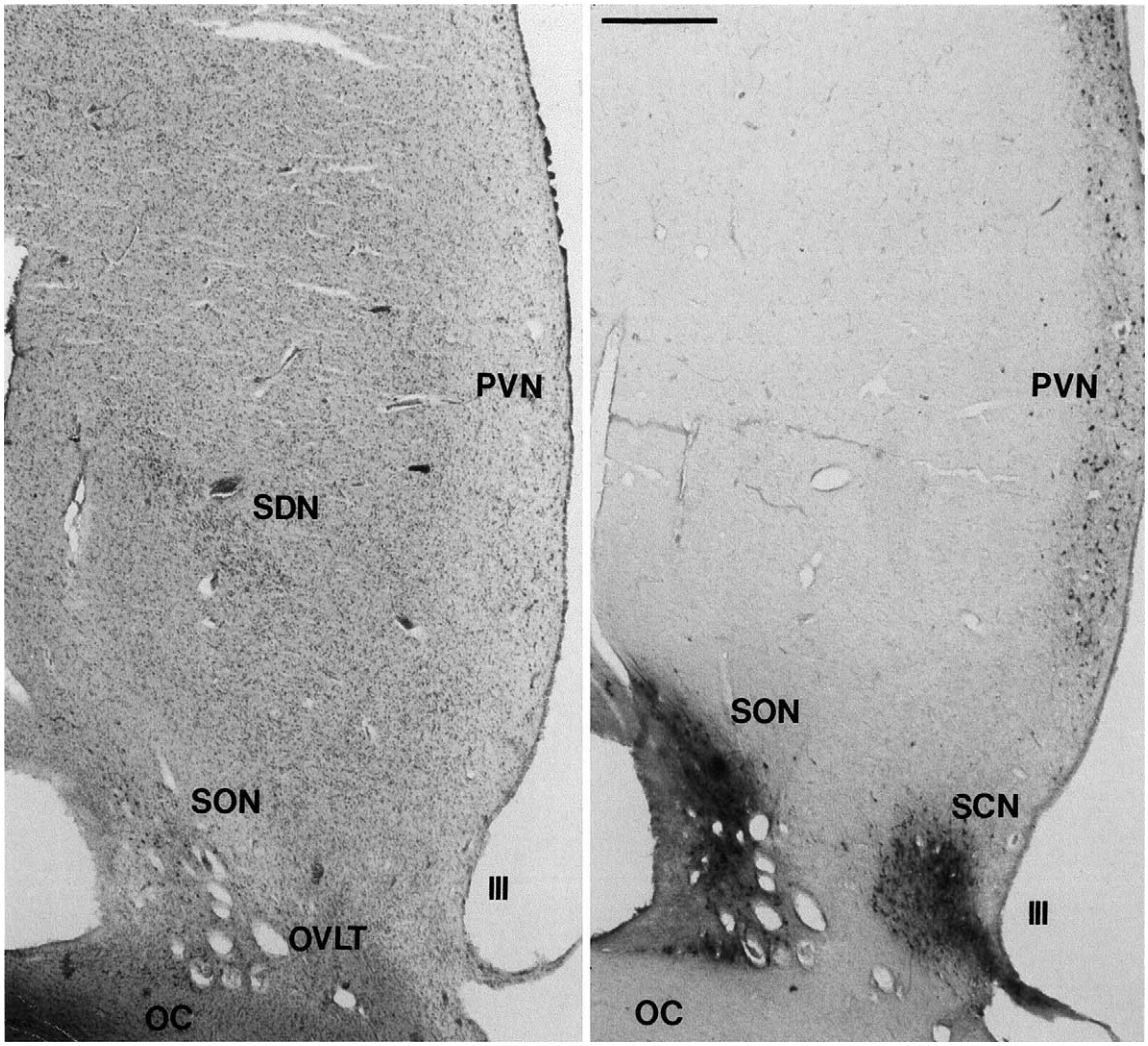

Fig. 2. Thionine (left)- and anti-vasopressin (right)-stained sections through the chiasmatic or preoptic region of the hypothalamus. OC, optic chiasm; OVLT, organum vasculosum lamina terminalis; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SDN, sexually dimorphic nucleus of the preoptic area; SON, supraoptic nucleus; III, third ventricle. Bar represents 1 mm.

[61], which also pointed to functional sex differences in this nucleus. Indeed, sex differences have been reported in sleep patterns that may be related to SCN sex differences. Women have higher percentages of slow-wave sleep and lower percentages of stage 1 sleep than men [111]. Women have about twice as many sleep spindles as men and tend to spend more time sleeping than men in a free-running environment. In addition, middle-aged women display more slow-wave sleep than middle-aged men. The period of free-running circadian rhythm is shorter and the fraction of sleep is significantly larger in women than in men [112]. In addition, testosterone has relatively specific and discrete effects on sleep and hormonal rhythms in men [66]. In healthy elderly women and men differences in entrained circadian temperature rhythms and sleep patterns exist that indicate that aging may affect the circadian timing system in a sexually dimorphic way [17,72]. Animal experiments indicate that only part of the sex differences in paradoxical sleep are dependent on circulating hormones [32].

3.2. The SCN, sexual behavior and reproduction

In addition to its possible involvement in reproduction, the SCN might also play a role in sexual orientation. In fact, the first difference in the human brain in relation to sexual orientation was observed in the SCN [102]. Morphometric analysis of the SCN of 10 homosexual men revealed that the volume of this nucleus was 1.7 times larger than that of a reference group of 18 presumed heterosexual men, and that it contained 2.1 times as many cells (Fig. 8). In fact, the same high number of SCN vasopressin neurons as observed in 1–2-year-old children [104] were also found in homosexual men [102]. It seems, therefore, as if the programmed postnatal cell death, which appears to occur in the SCN

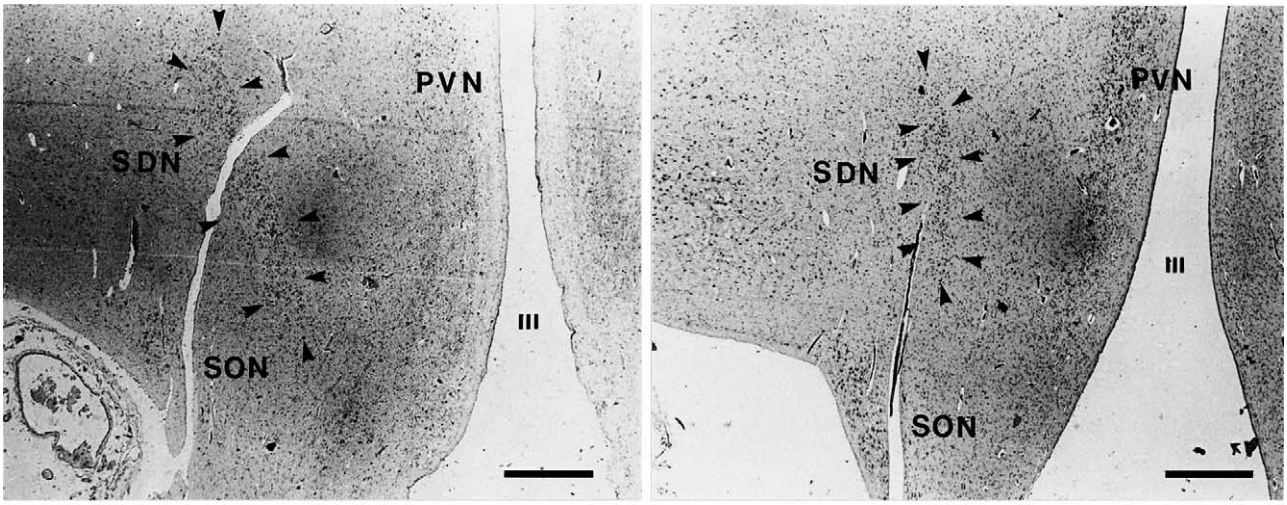

Fig. 3. Thionine-stained frontal section (6 μm) of the hypothalamus of (A) a 28-year-old man and (B) a 10-year-old girl. Arrows show the extent of the SDN-POA. Note the large blood vessel penetrating the SDN-POA and note that the SDN of the man is larger than that of the girl (from [99], with permission).

between 13 and 16 months after birth does not occur to the same extent in homosexual men. The increased number of vasopressin-expressing neurons in the SCN of homosexual men appeared to be quite specific for this subgroup of SCN neurons, since the number of VIP-expressing SCN neurons was not changed. However, in both the vasopressin and VIP neurons in the SCN, a reduced nuclear diameter was observed in homosexual men, suggesting metabolic alterations in the SCN in relation to sexual orientation [116].

A number of experimental data and observations on human material indicate that the SCN is involved in aspects of sexual behavior and reproduction. Already in the early seventies post-coital ultrastructural changes indicating neuronal activation were reported in the SCN of the female rabbit [20]. Important is also that the metabolic activity of SCN neurons, as judged from the nucleolar size, increases suddenly around puberty [7], indicating the addition of a reproductive function to the already mature circadian functions of the SCN. In addition, SCN efferents innervate the preoptic area that is involved in reproductive behavior. Furthermore, extensive lesioning of the SCN area results in failure of ovulation in the female rat [13]. The ovarian reproductive cycle is controlled

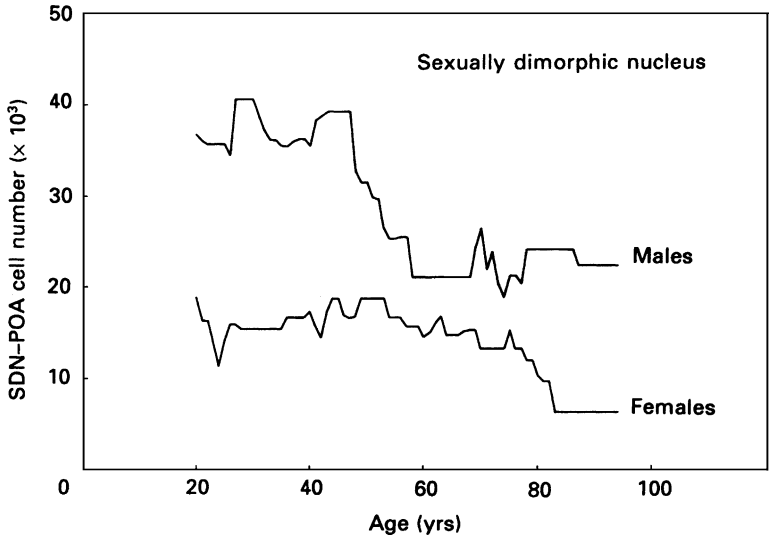

Fig. 4. Age-related changes in the total cell number of the sexually dimorphic nucleus of the preoptic area (SDN-POA) in the human hypothalamus. The general trend in the data is enhanced by using smoothed growth curves. Note that in males, SDN-POA cell number declines steeply between the ages of 50 and 60 years, whereas in females, from the age of about 50 years, a more gradual cell loss is observed, which continues up to old age. These growth curves demonstrate that the reduction in cell number in the human SDN-POA in senescence is a non-linear, sex-dependent process (from [49], with permission).

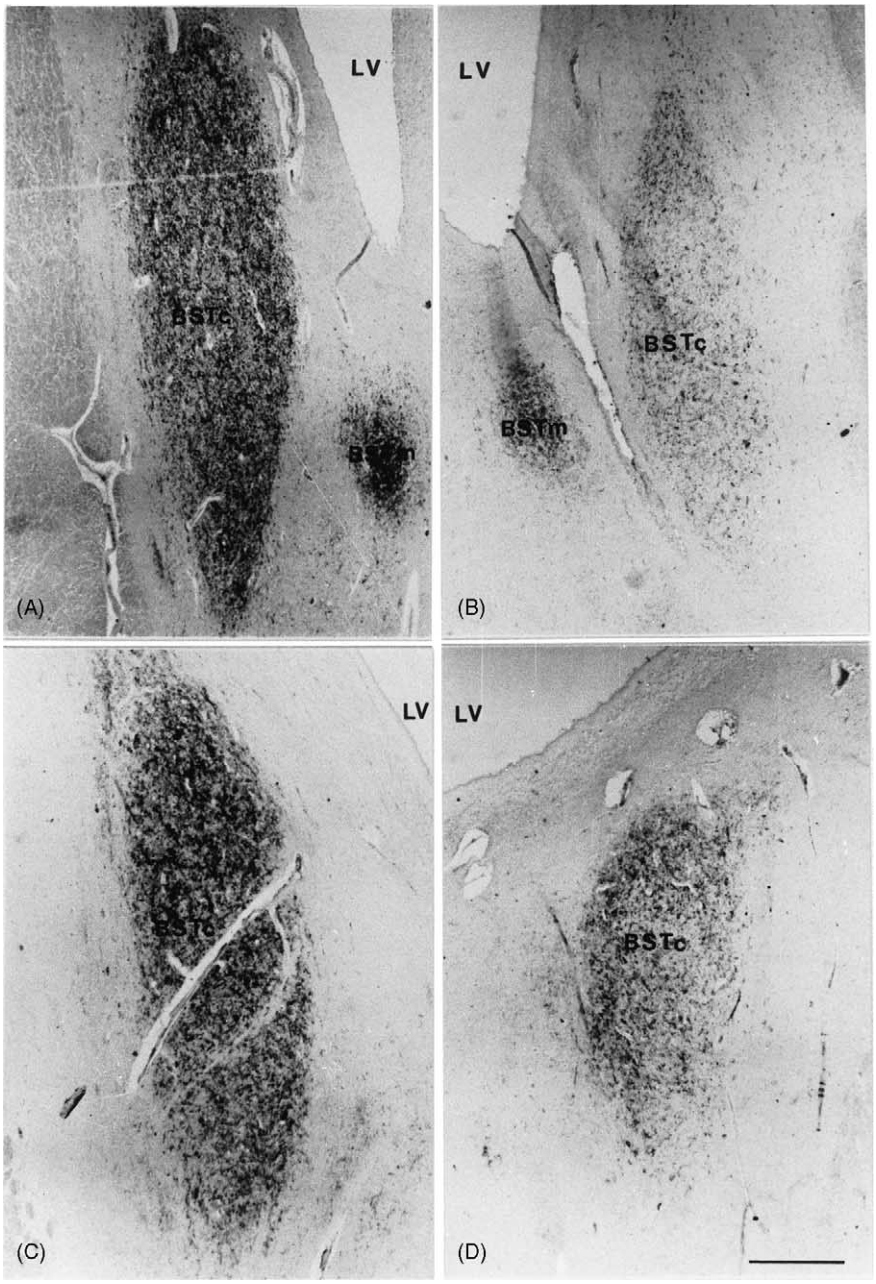

Fig. 5. Representative sections of the BSTc innervated by vasoactive intestinal polypeptide (VIP). (A) Heterosexual man; (B) heterosexual woman; (C) homosexual man; (D) male-to-female transsexual. Scale bar, 0.5 mm. LV, lateral ventricle. Note there are two parts of the BST in (A) and (B): small medial subdivision (BSTm) and large oval-sized central subdivision (BSTc). A female volume was observed in genetically male (male-to-female) transsexuals. There was no difference according to sexual orientation (from [117], with permission).

by the SCN, probably through a direct monosynaptic innervation of luteinizing-hormone-releasing-hormone (LHRH) neurons by VIP fibers [108,109]. Several morphological sex differences in the SCN have been reported that support its putative role in regulating reproductive functions. The SCN of male rats contains a larger amount of axo-spinal synapses, postsynaptic density material, asymmetrical synapses and their neurons contain more nucleoli than those of female rats [45,46]. The sex difference in synaptic number in the rat SCN depends on androgens in development [65]. In gerbils, the volume of the SCN is sexually dimorphic [50] and so is the organization of astroglia in the SCN [22].

The human SCN contains sex hormone receptors [61] and a sex difference was found both in the shape of the vasopressin subdivision of the human SCN [101] and in the number of VIP-containing neurons. The number of VIP-expressing neurons in the SCN is larger in men of 10–40 years and larger in women of 41–65 years of age [97,116]. The human SCN neurons contain staining for ERα, β and progesteron. A stronger ERα expression was

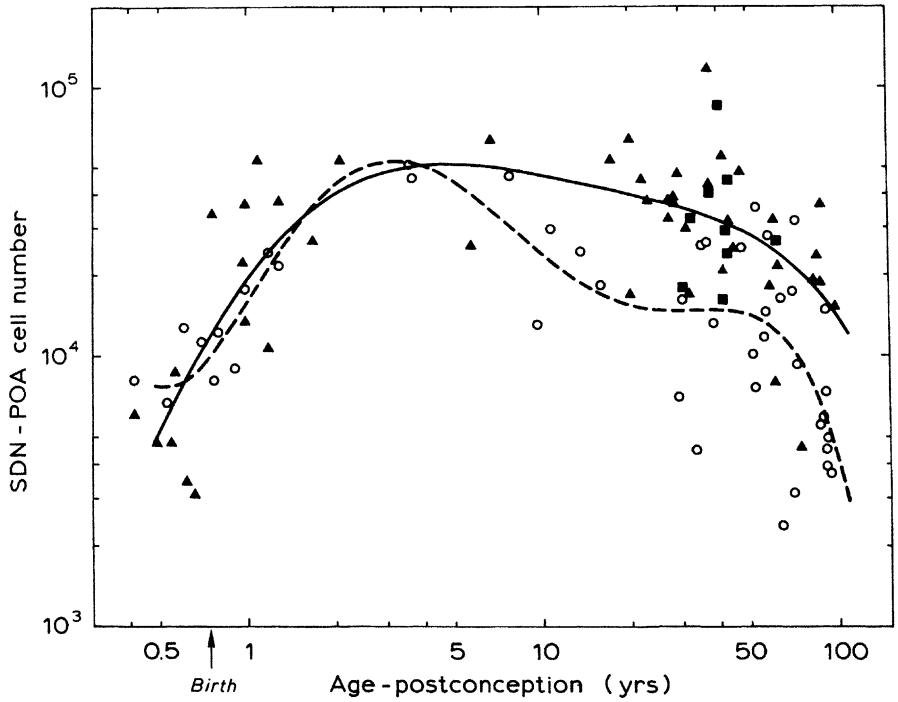

Fig. 6. Developmental and sexual differentiation of the human sexually dimorphic nucleus of the preoptic area (SDN-POA) of the preoptic area of the hypothalamus in 99 subjects, log–log scale. Note that at the moment of birth, the SDN-POA is equally small in boys (▲) and girls (○) and contains about 20% of the cell number found at 2–4 years of age. Cell numbers reach a peak value around 2–4 years postnatally, after which a sexual differentiation occurs in the SDN due to a decrease in cell number in the SDN of women, whereas the cell number in men remains approximately unchanged up to the age of 50. The SDN-POA cell number in homosexual men (■) does not differ from that in the male reference group. The curves are quintic polynomial functions fitted to the original data for men (full line) and women (dashed line) (from [100], with permission).

found in the SCN of females compared to males [61]. These observations are also consistent with sexually dimorphic functions of the SCN that must, however, still be better defined.

In seasonal breeders, VIP immunoreactivity in the SCN changes in relation to seasonal fluctuations in sexual activity [63]. The activation of c-fos in the SCN by sexual stimulation also points to a role of the SCN in reproduction [77]. Bakker et al. [11] have found that male rats treated neonatally with the aromatase inhibitor ATD show a clear sexual partner preference for females when tested in the late dark phase. When tested in the early dark phase, however, they showed a lesser preference for the female, or no preference at all. This is the first experimental indication of the involvement of the clock, i.e. the SCN, in sexual orientation. The number of vasopressin-expressing neurons in the SCN of these ATD-treated bisexual animals was increased [103], which was also found in homosexual men [102]. This observation supports the possibility that the increased number of vasopressin-expressing neurons in the SCN of adult homosexual men reflects a difference in the early stages of development in the brain.

A prominent theory is that sexual orientation develops as a result of an interaction between the developing brain and sex hormones [28,39]. According to Dörner's hypothesis, homosexual men would have a female differentiation of the hypothalamus. Although LeVay's [67] data on the small female-sized INAH-3 in homosexual men are in agreement with this theory, this idea was not supported by our data on the SDN-POA and central nucleus of the bed nucleus of the BSTc in homosexual men. Neither the SDN-POA or BSTc volume nor cell number differed in homosexual men who died of AIDS from that of the male reference groups in the same age range, nor from that of heterosexuals also suffering from AIDS ([59,100,117]; Fig. 8). The fact that no difference in SDN-POA or BSTc cell number was observed between homo- and heterosexual men, and the large SCN found in homosexual men [102] refutes the general formulation of Dörner's [28] hypothesis that homosexual men would have "a female hypothalamus" and rather favors the idea that homosexual men are a "third sex", i.e. different from heterosexual men and women.

4. Aging and sex differences

The sexually dimorphic pattern in aging of the SDN-POA has been discussed above.

In the mediobasal hypothalamus of aged subjects, a striking sex difference has been reported in neurofibrillary pathology associated with abnormally phosphorylated tau protein. The pathology in the median eminence and

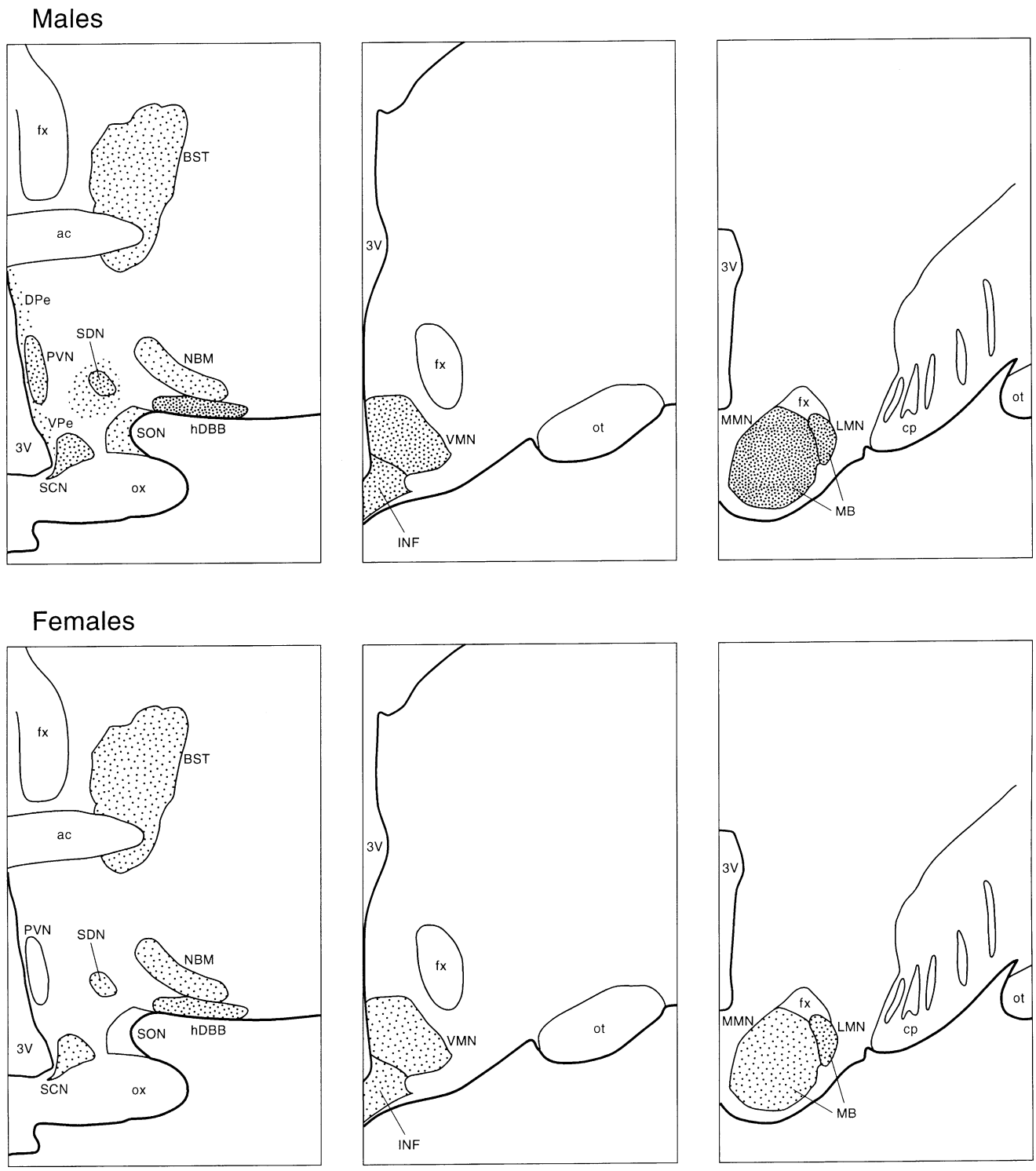

Fig. 7. Schematic representation of the sex differences in the intensity of androgen receptor immunoreactivity in the human hypothalamus. The three different sections correspond to plates 22, 27 and 30 of Mai et al. [69] human brain atlas. Abbreviations: ox, optic chiasma; NBM, nucleus basalis of Meynert; hDBB, horizontal limb of the diagonal band of Broca; SDN, sexually dimorphic nucleus of the preoptic area; SCN, suprachiasmatic nucleus; BST, bed nucleus of the stria terminalis; PVN, paraventricular nucleus; SON, supraoptic nucleus; Dpe, periventricular nucleus dorsal zone; Vpe, periventricular nucleus ventral zone; fx, fornix; 3V, third ventricle; ac, anterior commissure; VMN, ventromedial hypothalamic nucleus; INF, infundibular nucleus; ot, optic tract; MB, mamillary body, i.e. MMN, medial mamillary nucleus + LMN, lateromamillary nucleus; cp, cerebral peduncle (from [34], with permission).

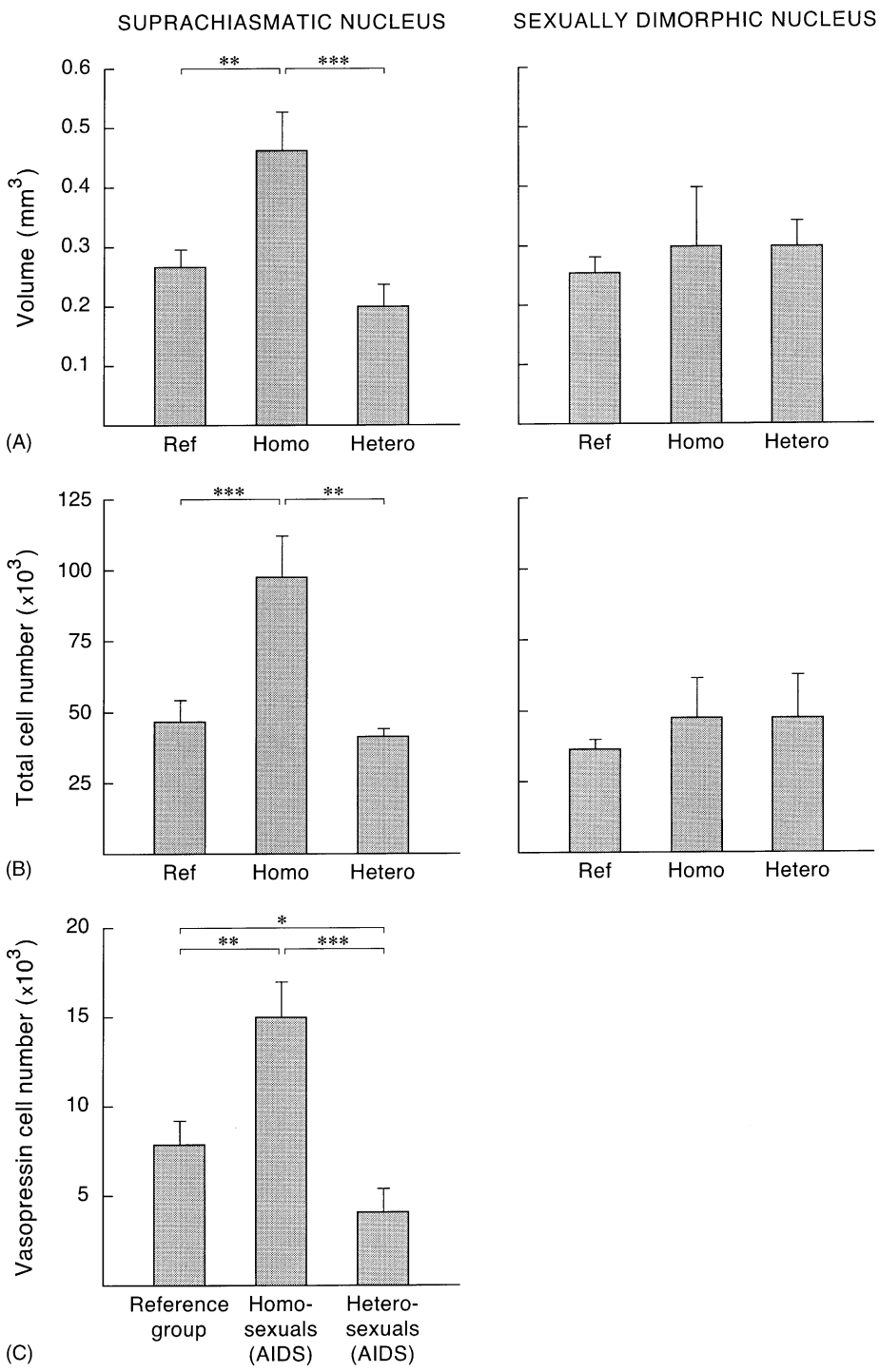

Fig. 8. (A) Volume of the human suprachiasmatic nucleus (SCN) and sexually dimorphic nucleus of the preoptic area (SDN) as measured in three groups of adult subjects: (1) a male reference group ($n = 18$); (2) male homosexuals who died of AIDS ($n = 10$); (3) heterosexuals who died of AIDS ($n = 6$; four men and two women). The values indicate medians and the standard deviation of the median. The differences in the volume of the SCN between homosexuals and the subjects from both other groups are statistically significant (Kruskal–Wallis multiple comparison test, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). Note that none of the parameters measured in the SDN (A and B) showed significant differences among the three groups (P always >0.4). (B) Total number of cells in the human SCN and SDN. The SCN in homosexual men contains 2.1 times as many cells as the SCN in the reference group of male subjects and 2.4 times as many cells as the SCN in heterosexual AIDS patients. (C) The number of vasopressin neurons in the human SCN (the SDN does not contain vasopressin-producing cells). The SCN in homosexual men contains, on average, 1.9 times as many vasopressin neurons as the SCN in heterosexual AIDS patients. Notice that the SCN of heterosexual individuals who died of AIDS contains fewer vasopressin cells than the SCN of the subjects from the reference group (from [102], with permission).

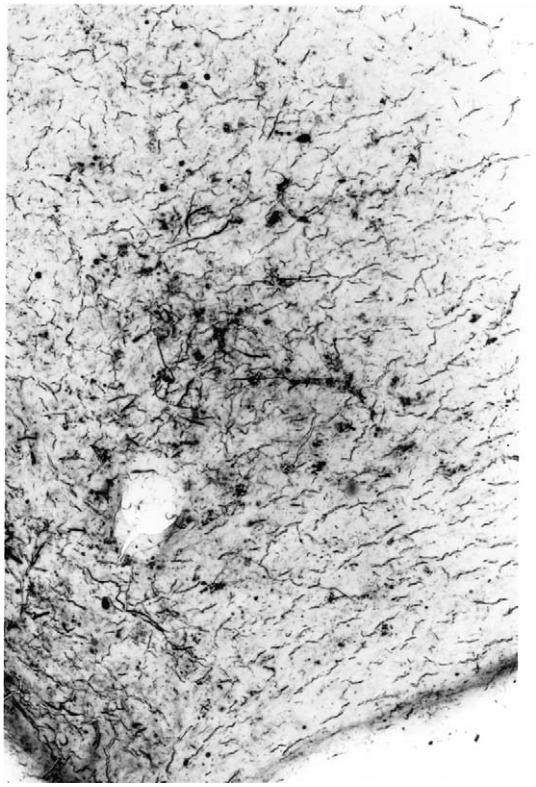

Fig. 9. Mediobasal hypothalamus including the infundibular nucleus of a 66-year-old male with advanced cytoskeletal pathology stained by Alz-50 for hyperphosphorylated tau. Such pathology is rarely present in postmenopausal women, which suggests that hyperactivity of neurons protects them against Alzheimer changes. Scale bar: 0.5 mm.

infundibular nucleus is characterized by a dense network of large dystrophic neurites with neurofibrillary tangles that are interspersed among them. The terminal-like processes contact the neurohaemal vasculature of the posterior median eminence and the adjacent infundibular nucleus (Fig. 9). The Alzheimer pathology in the infundibular nucleus was identified in up to 90% of the older males and in only 8–10% of the females. The vessel-associated neurofibrillary lesions of the mediobasal hypothalamus develop independently of Alzheimer's disease-related neocortical pathology [88–90]. In the arcuate nucleus of postmenopausal women, LHRH neurons and interneurons are strongly activated [80–82]. We propose that the lack of neurofibrillary changes in the mediobasal hypothalamus of females is an illustration of how activated neurons are protected against the development of Alzheimer changes, a principle we paraphrased as "use it or lose it" [96,105].

An opposite sex difference in Alzheimer pathology was observed in the nucleus basalis of Meynert (NBM), which is the major source of cholinergic innervation of the neocortex and which is severely affected in Alzheimer's disease. The percentage of NBM neurons containing pretangles with hyperphosphorylated tau was higher in females than in males. This sex difference may be related to the higher prevalence of Alzheimer's disease observed in women [87].

5. Functional sex differences in the hypothalamus as revealed from postmortem tissue

Males have higher vasopressin levels than females, even though the number of vasopressin neurons in the supraoptic nucleus (SON) did not differ between men and women [91,110]. This sex difference is explained by the higher activity we found in vasopressin neurons in the SON of young males as compared to females using the size of the Golgi apparatus as a measure for neuronal activity. In the course of aging, possibly triggered by the decrease in estrogen levels in postmenopausal women, the neuronal activity in the SON gradually increases in females, while it remains stable in males. The sex difference in neuronal activity in the SON thus disappears after the age of 50 [54]. Consequently, this is an example of a hypothalamic system that shows no structural sex difference but a functional sex difference instead. It is also an example of a sex difference based on the "activating" (or in this case "inhibiting") effect of sex hormones. The activation of neurosecretory vasopressin neurons in postmenopausal women was confirmed by measurement of the cell size as a parameter for neuronal activity in immunocytochemically stained vasopressin neurons. Vasopressin neurons in the SON and paraventricular nucleus (PVN) of the hypothalamus appeared to be larger in young men than in young women. In elderly women (>50 years old) vasopressin cell size considerably exceeded that of young women. In addition, vasopressin cell size correlated positively with age in women, but not in men in both nuclei. Sex differences in the size of the PVN vasopressin neurons were pronounced on the left side and absent on the right, indicating the presence of functional lateralization of this nucleus. These data demonstrate sex differences in the size of the vasopressin neurons, and thus in their function, that are age-dependent and probably also lateralized. No such changes were observed in oxytocin neurons in the PVN [55]. Sex- and age-related differences in the activity of vasopressin neurons in the human SON are probably mediated by differences in ERα and β expression by these cells. Young women (≤50 years old) show 50 times more ERβ nuclear positive vasopressin neurons than young men and 250 times more than postmenopausal women. By contrast, ERα is present in a higher proportion of the SON cells in young men and elderly women than in young women. The activation of vasopressin neurons in postmenopausal women is thus probably mediated by a decrease in nuclear ERβ as a possible mediator of inhibitory effects of estrogens, and an increase in nuclear ERα as a possible mediator of stimulatory effects of estrogens in these neurons [56].

Another example of a sex difference based upon the activating effect of sex hormones was found in the mamillary body complex (MBC) that shows much stronger androgen

receptor staining in males than in females [34]. Electrical stimulation of this area in monkeys induces penile erections [68,79]. In a follow-up study we have shown that this sex difference depends on the amount of circulating androgens in adulthood, while the sex difference in androgen receptors did not seem to be related to sexual orientation or gender identity [60]. Together, these data support the notion that a number of sex differences in the adult human hypothalamus are related to circulating levels of sex hormones.

6. Transsexuality and other gender identity problems

Transsexuality is a rare condition. The annual incidence of transsexuality in Sweden has been estimated to be 0.17 per 100,000 inhabitants. The sex ratio (genetic male:female) varies from country to country between 1.4:1 and 3:1 [37,64]. There is only little information about the factors that may influence gender and cause transsexuality in humans (Table 2).

The disparate maternal aunt–uncle ratio in transsexual men has been hypothesized to be due to genomic imprinting [43]. There are only a few reports that have found chromosomal abnormalities in transsexuals. Six cases of male-to-female transsexuals with 47,XYY chromosome and one female-to-male transsexual with 47,XXX have been reported [108]. Moreover, transsexualism has been reported in a man with Kleinfelter (XXY) [86]. In addition, pairs of monozygotic female twins have requested sex reassignment and familial cases of gender identity problems were reported, suggesting a genetic basis for this disorder [44,86]. Although only a minority of the transsexuals has an underlying endocrine abnormality [70], there are some indications of a possible disorder of the hypothalamo–pituitary gonadal axis in some transsexuals that may have a basis in development, such as a high frequency of polycystic ovaries, oligomenorrhea and amenorrhea in female-to-male transsexualism [36,86] (Fig. 10).

Dessens et al. [25] reported that three children born of a group of 243 women exposed to the anticonvulsants phenobarbital and diphantoin were found to be transsexuals, while, in addition, there were a few other subjects with gender dysphoria/cross-gender behavior. Gender problems thus occurred remarkably often in view of the rarity of this disorder. This exciting observation on the effect of compounds that are known to alter steroid levels in animal experiments has to be examined further. In this respect it is of interest to note that phenobarbital has been widely used as prophylactic treatment in neonatal jaundice, and greatly elevated the postnatal rise in testosterone [35]. In 1996, Meyer-Bahlburg et al. reported a gender change from woman to man in four 46,XX individuals with classical congenital adrenal hyperplasia. Congenital adrenal hyperplasia, characterized by high androgen levels during prenatal development, indeed constitutes a risk factor for the development of gender identity problems. Although it should be emphasized that the large majority

Table 2
Factors that influence sexual differentiation of the human brain

Gender identity (transsexualism)	
Chromosomal disorders	Rare: 47,XYY (male-to-female), 47,XXX (female-to-male; [106]) Steroidogenic factor-1 mutation gives sex reversal, not transsexuality [1] Kleinfelter XXY male-to-female [86] Twin studies [24,86] Genomic imprinting [43]
Phenobarbital/diphantoin	[25]
Hormones	Intersex [83,121] Cloacal extrophy 5α-Reductase deficiency, 17β-hydroxy-steroid-dehydrogenase-3 deficiency [52,53,114] CAH girls with gender problems [71,121] More polycystic ovaries, oligomenorrhea and amenorhea are found in transsexuals [36] Complete androgen insensitivity syndrome results in XY heterosexual females [115] However, in congenital deficiency of oestrogens due to aromatase deficiency or oestrogen resistance, gender identity is not affected [18,33,76,85,92]
Social factors?	[12] not effective: John-Joan-John case [21,26]
Sexual orientation (homo/heterosexuality)	
Genetic factors	Twin studies [10,57] Molecular genetics [47,51]. However, see Rice et al. [84].
Hormones	CAH girls [27,75,121] DES [29,71] Male-to-female sex reassignment [9]
Chemicals	Nicotine prenatally increases the probability of lesbianism [31]
Social factors?	Stress during pregnancy [8,30,31] Raising by transsexual or homosexual parents does not affect sexual orientation [40,42]

For references see [98], if not in text.

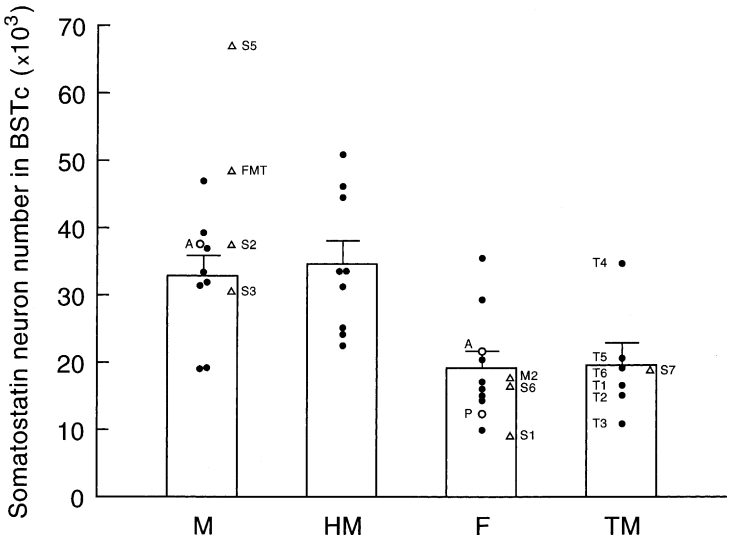

Fig. 10. BSTc neuron numbers. Distribution of the BSTc neuron numbers among the different groups according to sex, sexual orientation, and gender identity. M, heterosexual male reference group; HM, homosexual male group; F, female group; TM, male-to-female transsexuals. The sex hormone disorder patients S1, S2, S3, S5, S6 and M2 indicate that changes in sex hormone levels in adulthood do not change the neuron numbers of the BSTc. The difference between the M and the TM group ($P < 0.04$) is also statistically significant according to the sequential Bonferonni method if S2, S3, and S5 are included in the M group or if S7 is included in the TM group ($P \leq 0.01$). Note that the number of neurons of the FMT is fully within the male range. Whether the transsexuals were male oriented (T1, T6), female oriented (T2, T3, T5) or both (T4) did not have any relationship with the neuron number of the BSTc. The same holds true for heterosexual and homosexual men. This shows that the BSTc number of somatostatin neurons is not related to sexual orientation. A, AIDS patient. The BSTc number of neurons in the heterosexual man and women with AIDS remained well within the corresponding reference group (see Fig. 1), so AIDS did not seem to affect the somatostatin neuron numbers in the BSTc. P, postmenopausal woman S1 (♂ 46 years of age): adrenal cortex tumor for more than 1 year, causing high cortisol, androstendione, and testosterone levels. S2 (♂ 31 years of age): feminizing adrenal tumor that induced high blood levels of oestrogens. S3 (♂ 67 years of age): prostate carcinoma; orchiectomy 3 months before death. S5 (♂ 86 years of age): prostate carcinoma; prostatectomy; orchiectomy, and antiandrogen treatment for the last 2 years. S6 (♀ 25 years of age): Turner syndrome ([45], X0; ovarian hypoplasia). M2 (♀ 73 years of age): postmenopausal status (from [59], with permission).

of women with this disorder do not experience a marked gender identity conflict, the odds ratio that a genetic female with this disease would live, as an adult, in the male social role compared to genetic females in the general population was found to be 608:1 [119]. These observations support the view that intrauterine or perinatal exposure to abnormal levels of sex hormones may permanently affect gender identity.

The concept of sexual neutrality at birth after which the infant differentiates as masculine or feminine as a result of social experiences, was proposed by Money et al. [73]. Gender imprinting was presumed to start at the age of 1 year and to be already well established by 3–4 years of age [74]. Observations on children with male pseudohermaphroditism due to 5-α-reductase-2 deficiency were supposed to support the influence of life experience on psycho-sexual make-up [2]. A classic report which has strongly influenced the opinion that the environment plays a crucial role in gender development was the one described by Money of a boy whose penis was accidentally ablated at 8 months of age during a phemosis repair by cautery and who was subsequently raised as a girl. Orchiectomy followed within a year to facilitate feminization and further surgery to fashion a full vagina was performed later. Initially this individual was described as developing into a normally functioning woman. Later, however, it appeared that the individual had rejected the sex of raising and switched at puberty to living as a man again and he requested male hormone shots, a mastectomy and phalloplasty. At the age of 25, he married a woman and adopted her children. This famous John-Joan-John story, although it is just one case, illustrates that there is little, if any, support for the view that individuals are sexually neutral at birth and that normal psychosexual development is dependent on the appearance of the genitals [26]. In a second case of penile ablation in which the decision was made to reassign the patient as a girl and raise the baby as a girl, the remainder of the penis and testes were removed at a slightly earlier stage, at 7 months. Although her sexual orientation was bisexual and even though she was mainly attracted to women, her gender identity was female. The different outcome as compared with the former case is explained by the authors on the basis of the decision to reassign the sex at an earlier age [12].

Reiner [83] described a 46,XY child with mixed gonadal dysgenesis, one immature testis, hypoplastic uterus and clitoral hypertrophy, who was raised without stigmatization as a girl but who declared himself male at the age of 14. Following corrective surgery and testosterone substitution he lived as a boy despite the social factors that were clearly in favor of maintaining the assigned sex. Apparently the deficient testis had been able to organize the brain during development

even though the hormone levels were prenatally so inadequate that ambiguity of the genitalia was induced.

A child with true hermaphroditism, 45,X (13%) 47,XYY (87%) sex chromosome mosaic pattern in blood, uterus, fallopian tubes, phallus, testicular tissue and epididymis, was assigned the male sex at birth. At 5 weeks, the decision was made to reassign him to female. At 9 months, an operation was performed to make the genitalia female, at 13 months the testicle was removed and at the age of 5 another operation was done to make the genitalia female. She was raised as a girl, but had masculine interests and when she was around 8 years of age she declared that 'God had made a mistake' and that she 'should have been a boy'. Apparently the male sex hormones to which she had been exposed in utero had imprinted the male gender, although the authors also presumed postnatal psychosocial factors to have played a role [118].

As described above, we recently found a female-sized central nucleus of the bed nucleus of the BST in male-to-female transsexuals ([117]; Fig. 5). These data were confirmed by neuronal counts of somatostatin cells, the major neuron population in the BSTc [59] (Fig. 10). Changes in adult hormone levels could not explain this difference. These observations support the hypothesis that gender identity develops as a result of an interaction between the developing brain and sex hormones. Much to our surprise, however, the sex difference in BSTc volume did not become overt until adulthood [19]. The explanation for the discrepancy between the late occurrence of a sex difference in the volume of this nucleus and the early occurrence of gender problems in transsexualism necessitates further research. Functional sex differences in the BSTc may precede the structural sex differences in the course of development.

7. Homosexuality

Sexual orientation is influenced by quite a number of genetic as well as non-genetic factors (Table 2). Genetic factors appear from studies in families, twins and through molecular genetics [9,10,47,51,57,78,107]. Hamer and co-workers found linkage between DNA markers on the X-chromosome and male sexual orientation. Genetic linkage between the microsatellite markers on the X chromosome, i.e. Xq28, was detected for the families of gay males, but not for the families of lesbians [47,51]. In a follow-up study, Rice et al. [84] studied the sharing of alleles at position Xq28 in 52 Canadian gay male sibling pairs. Allele and haplotype sharing for these markers was not increased more than expected, which did not support the presence of an X-linked gene underlying male homosexuality. In a reaction to this paper, Hamer [48] stated that (i) the family pedigree data from the Canadian study supported his hypothesis, (ii) that three other available Xq28 DNA studies found linkage and (iii) that the heritability of sexual orientation is supported by substantial evidence independent of the X-chromosome data. In a meta-analysis of the four available studies he found a significant linkage. Rice et al. [84] responded extensively and remained convinced that an X-linked gene could not exist in the population with any sizeable frequency. This controversy will undoubtedly continue.

Sex hormones during development also have an influence on sexual orientation, as appears from the increased proportion of bi- and homosexual girls with congenital adrenal hyperplasia [27,71,75]. Then there is diethylstilboestrol (DES), a compound related to estrogens that increases the occurrence of bi- or homosexuality in girls whose mothers received DES during pregnancy [29,71] in order to prevent miscarriage (which it does not do). Whether environmental estrogens, e.g. from plastics can influence sexual differentiation of the human brain and behavior is, at present, in debate but certainly not established. In addition, phytoestrogens, such as resveratrol, present in grapes and wine and an agonist for the estrogen receptor should be considered in this respect [38].

Prenatal nicotine exposure has masculinizing/defeminizing effects on sexual orientation of female offspring and increases the probability of lesbianism [31].

Maternal stress is thought to lead to increased occurrence of homosexuality in boys, particularly when the stress occurs during the first trimester [30,31], and in girls [8]. As an interesting case history of this potentially environmental factor, Weyl [113] has mentioned that Marcel Proust's mother was subjected to the overwhelming stress of the Paris commune during the fifth month of her pregnancy in 1871 and that Mary, Queen of Scots, the mother of the homosexual king of England, James I, toward the end of the fifth month of pregnancy had the terrifying experience that her secretary and special friend Riccio was killed. Although postnatal social factors are generally presumed to be involved in the development of sexual orientation [15], solid evidence in support of such an effect has not yet been reported. The observation that children raised by lesbian couples or by transsexuals generally have a heterosexual orientation [40,42,58] does not support the possibility of the social environment in which the child is raised as an important factor for determining sexual orientation, nor is there scientific support for the idea that homosexuality has psychoanalytical or other psychological or social learning explanations, or that it would be a 'lifestyle choice" [30]. Various hypothalamic structures are structurally different in relation to sexual orientation, i.e. the suprachiasmatic nucleus [102], INAH-3 ([67]; not confirmed by Byne et al. [16] and the commissura anterior [6]; not confirmed by Byne et al. [16]), suggesting that a difference in hypothalamic neuronal networks that occurs in development may be the basis of differences in sexual orientation.

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